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Leaf Morphology of the Rheophyte, *Rhododendron indicum* f. *otakumi* (Ericaceae)

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Rhododendron indicum f. *otakumi* is an endemic rheophyte on Yakushima, Japan. The morphology and anatomy of the leaves of *R. indicum* f. *otakumi* and *R. indicum* f. *indicum*, its putative ancestor, were examined to determine differences between them. The leaves of f. *otakumi* are narrower than those of f. *indicum*, with leaf length to width ratios of 5.10 and 3.38, respectively. Our examination revealed that stenophylly in *R. indicum* f. *otakumi* was due to a decrease in the number of cells across the width of the leaf in both the adaxial epidermis and palisade tissues, even though the leaf cells of the epidermis and palisade tissue were larger in *R. indicum* f. *otakumi*. The heavy rainfall on Yakushima may have been a factor in the evolution of the narrow leaved form of *R. indicum*.

Key words: anatomy, leaf, rheophyte, *Rhododendron indicum* f. *indicum*, *Rhododendron indicum* f. *otakumi*

Rhododendron indicum (L.) Sweet f. *otakumi* T. Yamaz., endemic to the Otakumi River on Yakushima, Japan, is a rheophyte characterized by linear leaves and a deeply incised corolla that is usually separated into five petals (Yamazaki 1966, 1996). The flowers of *R. indicum* f. *otakumi* appear choripetalous. *Rhododendron indicum* f. *otakumi* is suspected of being derived from *R. indicum* f. *indicum*, an obligate rheophyte adapted to river-bank environments. Forma *indicum* occurs on Yakushima and along several river systems on Kyushu and Honshu, Japan (Yamazaki 1989, 1996). Forma *otakumi* is extinct in the wild, but is cultivated by the local government of Yaku-cho.

The narrow leaves of *Rhododendron indicum* f. *otakumi* are a typical adaptation to flooding according to van Steenis (1981), enabling plants to withstand swiftly flowing water after heavy rain. Forma *otakumi* is suspected of being derived from forma *indicum* in response to flooding on Yakushima, where annual rainfall is 4000 mm/yr on average and 7000 mm/yr at maximum (Takahara & Matsumoto 2002). In this study, we investigated the leaf characteristics of *R. indicum* f. *otakumi* and f. *indicum* to determine differences between them.

The leaves of rheophytes are generally thicker and narrower than those of their corresponding taxa in non-rheophytic environments. The ratio of blade length to width (leaf index) was determined to be 4 or greater in rheophytes (van Steenis 1981). The leaf morphology and anatomy of rheophytes and their non-rheophytic relatives have been studied previously by Kato & Imaichi (1991), Imaichi (1992), Kato (1992), Imaichi & Kato (1992), Kato & Imaichi (1992), Hiyama et al. (1992), Usukura et al. (1994) and Tsukaya (2002a). In rheophytic angiosperms, narrow leaves have been attributed to a decrease in the number of cells across the width of the lamina (Usukura *et al.* 1994) or to a decrease in the number and size of the cells (Tsukaya 2002a). Usukura *et al.* (1994) found in *Farfugium japonicum* a rough tendency for the narrower leaves of rheophytes to have a thicker cuticular layer, but with the size of the mesophyll cells and the volume of intercellular space differing only slightly from the broader leaves of the facultative rheophyte. Tsukaya (2002a) compared the leaves of the rheophyte *Dendranthema yoshinaganthum* and the non rheophytic *D. indicum* and the complex of hybrids between them and found that variation in leaf width among the taxa was due mostly to variation in the number of cells per leaf, with variation in cell size in the palisade tissue also playing a role. In this study, we compared the morphology and anatomy of two forms of *Rhododendron indicum* to determine the evolutionary changes brought about through adaptation to a rheophytic habitat.

Materials and Methods

Leaf samples of *Rhododendron indicum* f. *otakumi* were obtained from cultivated stock managed by the Yaku-cho government on Yakushima. Leaf samples of *R. indicum* f. *indicum* were collected from plants in the natural habitat on Yakushima. Collection dates were May 2000, June 2002 and January 2003. We collected one shoot with fully matured leaves from each individual. Ten shoots of f. *otakumi* and eight shoots of f. *indicum* were used. The shoots were fixed in FAA for morphological study. Voucher specimens (*H. Setoguchi J9943* and *G. Kajimaru 11 for R. indicum* f. *otakumi*, and *H. Setoguchi J9945* and *G. Kajimaru 12* for *R. indicum*) were deposited in the herbarium of Kyoto University (KYO).

Morphological and anatomical studies

All leaves, regardless of size, were measured using an electron caliper square. The leaf index was calculated as the ratio of blade length to blade width (van Steenis 1981). Leaves of rheophytes are usually glabrous or almost glabrous (van Steenis 1981). The hairs on the adaxial surface of the leaf were counted from photographs of the leaves. The density of hairs per unit square was also calculated. The leaves were measured at their thickest point. To determine the number and size of the adaxial epidermal and palisade cells in transverse sections of the blade at its widest point we used only mature leaves.

Results

Morphology

The blades of *Rhododendron indicum* f. *indicum* and f. *otakumi* were measured at various developmental stages to determine their length and width (see Fig. 1). There was a positive correlation between the length and width of the blade in both taxa (Fig. 2). The maximum width and length of the leaf blade of f. *otakumi* was ca. 3.5 mm and 17 mm, respectively. The leaf blades of f. *indicum* were larger in both dimensions; ca. 9.5 mm wide and 30 mm long. The leaf index of f. *otakumi* was determined to be 5.10 ± 1.07 while the leaf index of f. *indicum* was determined to be 3.38 ± 1.02 (Table 1).

The density of hairs on the adaxial leaf surface was determined for various developmental stages. There was a negative correlation between the density of hairs and the developmental stage of the leaf (determined by length and width of the lamina) in each taxon (Figs. 3, 4). The hairs were always less dense in f. *otakumi* than in leaves of the same width and/or length of f. *indicum*.

The variation in leaf thickness was determined at various developmental stages in f. *otakumi* and f. *indicum* (Fig. 5, 6). There was a positive correlation between thickness and length (Fig. 5) and between thickness and width of the blades (Fig. 6) in both

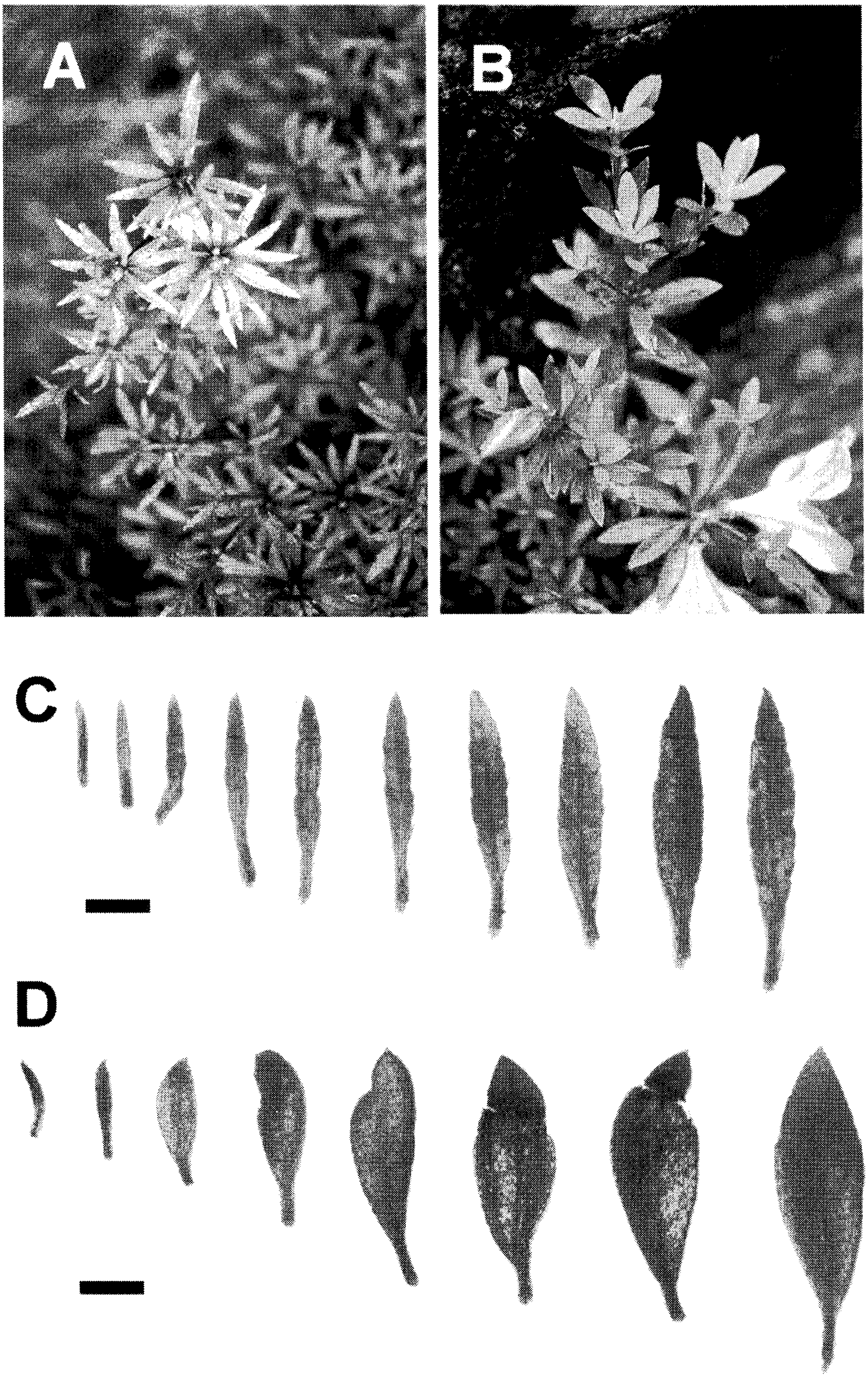

FIG. 1. A-D. Gross morphology of shoots and leaves. A: Gross morphology of shoots of *Rhododendron indicum* f. *otakumi*. B: Gross morphology of shoots of *R. indicum*. C: Leaves from a shoot of *R. indicum* f. *otakumi*. D: Leaves from a shoot of *R. indicum* f. *indicum*. Bars in C and D 5 mm.

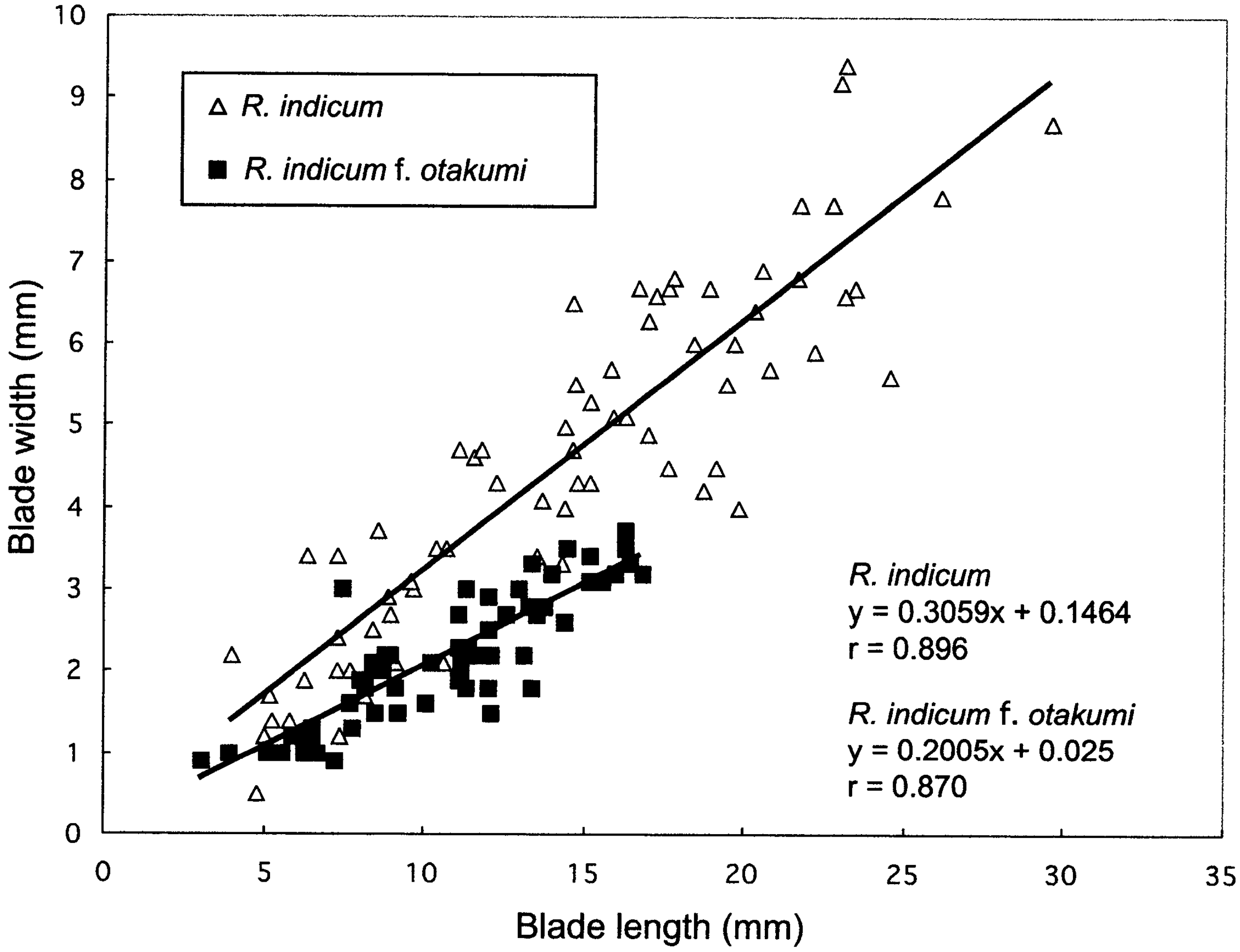

FIG. 2. Correlation between blade length and blade width. r is the correlation coefficient

taxa. The blades of f. *otakumi* were always thicker than those of f. *indicum* of the same width and/or length.

Anatomy

The number of adaxial epidermal cells in the leaf blades of f. *indicum* and f. *otakumi* were determined from observations of cross sections of mature leaves. The leaves of f. *otakumi* consistently had fewer epidermal cells than those of f. *indicum*, ca. 87 cells in *R. indicum* f. *otakumi* and ca. 197 cells in f. *indicum* across the width of the leaf (Table 2). The epidermal cells of the stenophyllous leaves of f. *otakumi* are slightly larger than those of the broader leaves of f. *indicum*. The palisade cells of f. *otakumi* are slightly longer than those of f. *indicum* in the leaf width direction. On the other hand, the palisade cells of f. *otakumi* are slightly shorter than those of f. *indicum* in leaf-thickness direction.

TABLE 1. Leaf indices and number of leaves examined in the present study.

Plant taxa	Leaf index (Mean ± SD)	Number of leaves examined
Rhododendron indicum	3.38 ± 1.07	71
R. indicum f. *otakumi*	5.10 ± 1.02	62

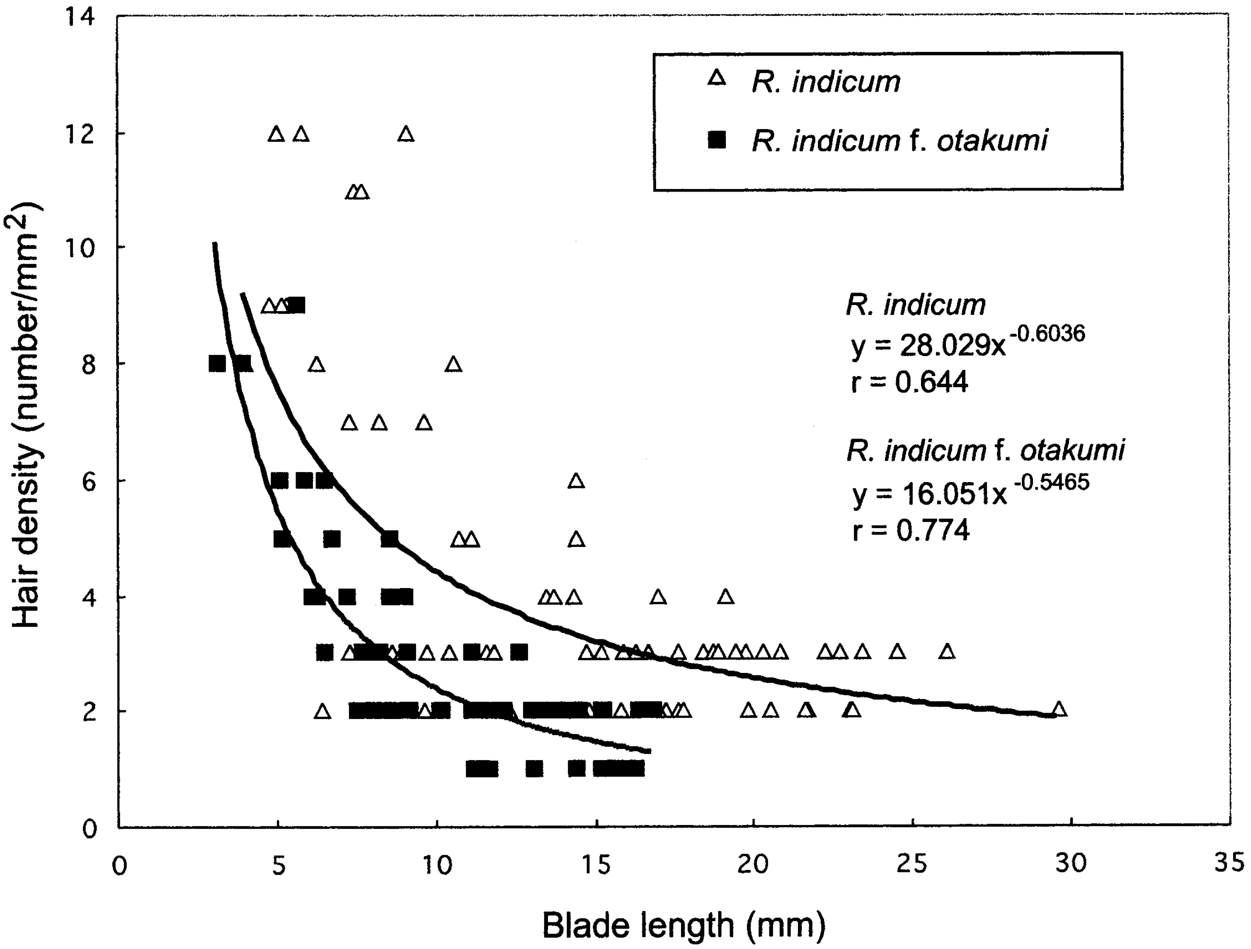

FIG. 3. Correlation between hair density and blade length. r is the correlation coefficient

Discussion

Rhododendron indicum f. *otakumi* and *Rhododendron indicum* f. *indicum* differ distinctly in leaf shape, especially in the width of the blade. The leaf index of *R. indicum* f. *otakumi* was greater than 5, indicating a typical stenophyllous, rheophytic leaf. The leaf index of f. *indicum* was 3.38, or less than the standard value for rheophytic leaves of 4 as indicted by van Steenis (1981), even though f. *indicum* is generally accepted to be a typical rheophyte on rocky riverbanks and streambeds in Japan. The low value of the leaf index can be explained by the hypothesis that plants adapt to riverbank environments through small changes (microphyllous changes) from an ancestral form.

This study also clarified the morphological attributes of less dense hairs on the adaxial leaf surface and the thicker blade of *Rhododendron indicum* f. *otakumi*, which are concordant with the characteristics of rheophytes as described by van Steenis (1981). Such attributes would result in reduced hydraulic pressure on the blade in swiftly flowing water and increased strength of the blade; stenophyllous leaves are more tolerant of swiftly flowing water.

Rhododendron indicum f. *otakumi* appears to be derived through mutation(s), e.g., narrowing of the leaf, from *R. indicum* f. *indicum* within Yakushima. The eastern region of Yakushima along the Otakumi River system, the original locality for *R. indicum* f. *otakumi*, is characterized by high annual rainfall, from 4000 - 7000 mm/yr (Takahara & Matsumoto 2002). The stenophyllous mutant of

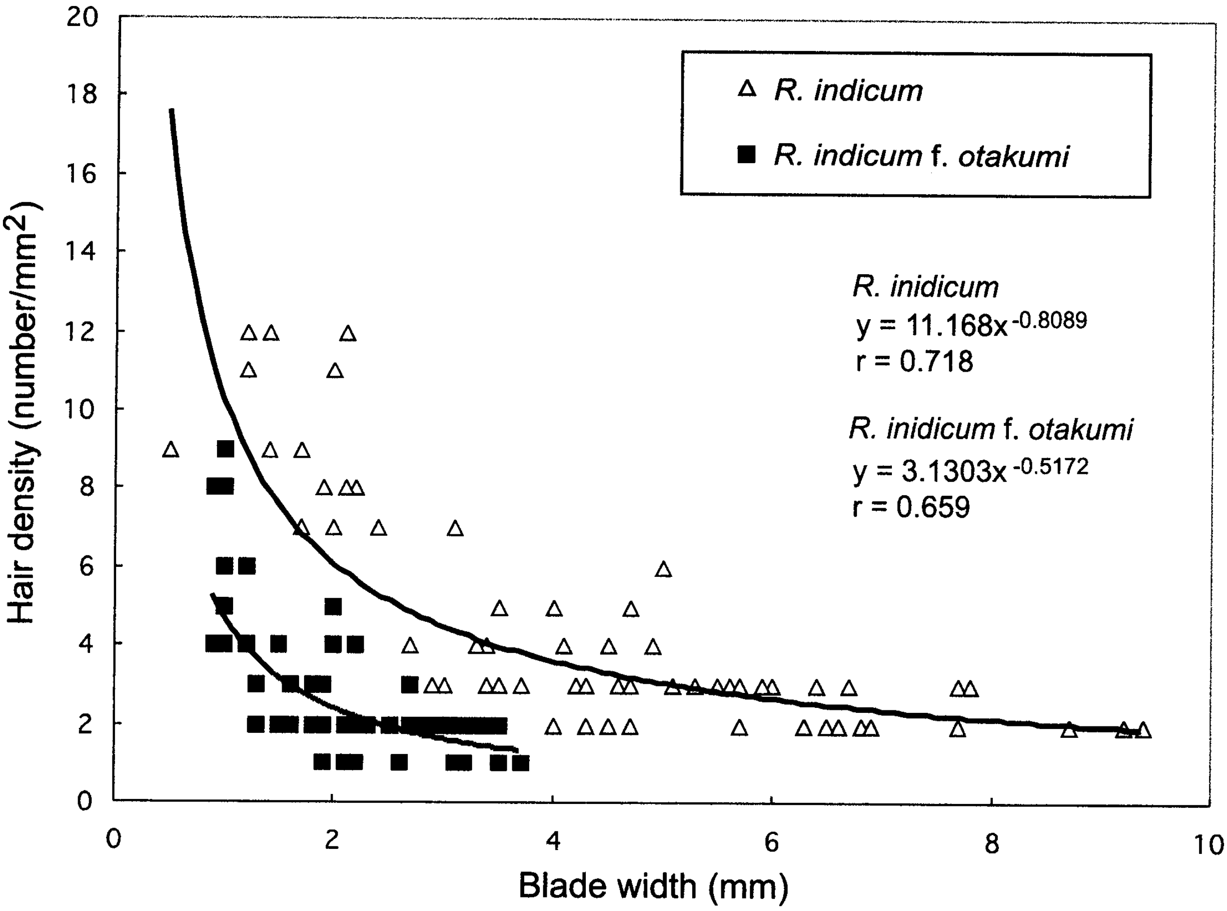

FIG. 4. Correlation between hair density and blade width. r is the correlation coefficient

f. *indicum* could survive in the environment even after decrease of potential for photosynthesis as a result of stenophyllous change (extinction of f. *otakumi* was mainly cased by the robbing of the gardening purpose).

The narrower leaves of *Rhododendron indicum* f. *otakumi* are due to fewer cells across the width of the leaf as compared to f. *indicum*. The number of adaxial epidermal cells across the width of the leaf is ca. 87 in f. *otakumi*, or less than half the number in f. *indicum* (ca. 196). The leaves of f. *otakumi* were less than half as wide as those of f. *indicum* (Fig. 1) even though the palisade cells across the width of the leaf were somewhat longer in the narrower leaves of f. *otakumi*. The number of palisade cells is clearly fewer in f. *otakumi*, although they were not counted directly.

The epidermal cells are slightly larger in f. *otakumi* than in f. *indicum*. The shape of the leaves of two related taxa as they relate to water flow on a riverbank was studied by Kato & Imaichi (1991), Imaichi & Kato (1992), Usukura *et al.* (1994) and Tsukaya (2002a). In the fern rheophyte, *Osmunda lancea*, the narrow leaves were attributed to a decrease in cell size and a decrease in the volume of intercellular space through heterochronic evolution. The number of adaxial epidermal and palisade cells was almost equal between the rheophyte and non rheophytic species. In the facultative rheophyte, *Farfugium japonicum* var. *luchuense*, leaf shape is correlated with the number of cells across the width of the leaf (Usukura *et al.* 1994). They found a rough tendency for the narrower leaves of rheophytes to have a thicker cuticular layer, but the

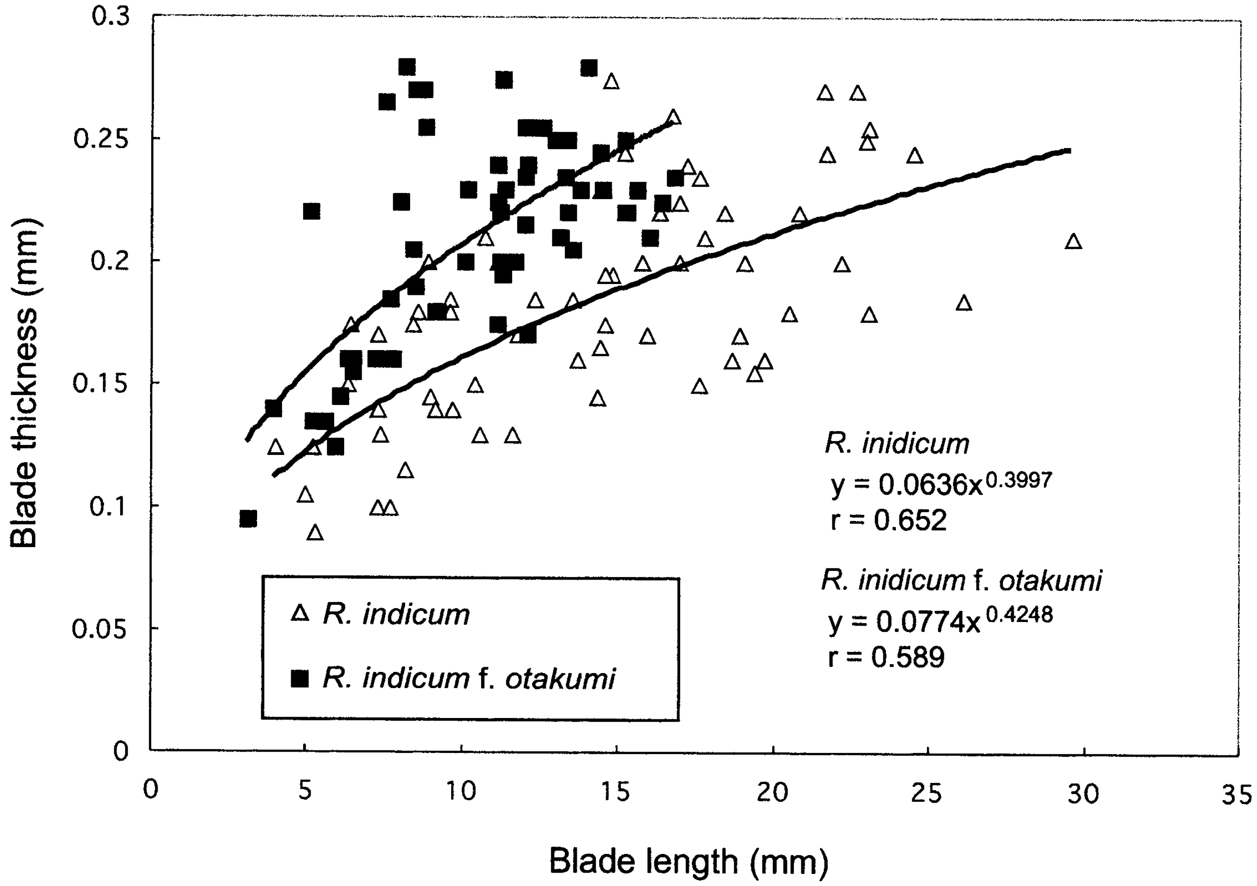

FIG. 5. Correlation between blade thickness and blade length. r is the correlation coefficient

size of the mesophyll cells and the volume of intercellular space differed only slightly in the facultative rheophyte. Tsukaya (2002a) reported that both the number and size of the palisade cells was correlated with the narrower leaves in the rheophyte *Dendranthema yoshinagathum*. In this study, a decrease in the number of cells in the adaxial epidermis and palisade layers was observed, but the adaxial epidermal cells and palisade cells showed a slight increase in size. These observations are summarized in Table 3. Clearly, land plants have adapted to a rheophytic environment along several different pathways.

The cells across the width of the leaf were slightly larger both in the epidermis and palisade layer. Stenophylly in *Rhododendron indicum* f. *otakumi* was therefore the result of a decrease in number of cells across the leaf. The palisade cells, however, were slightly shorter (Table 2), although the blades were actually thicker in the rheophyte. In *R. indicum* f. *otakumi*, therefore, the narrowing of the leaves appears to be governed by different mechanisms in the epidermis and palisade tissue.

Genetic evidence from examples of leaf mutations provides insights into the leaf morphology of *Rhododendron indicum* f. *indicum* and f. *otakumi*. The *Cdc2* overexpressor of tobacco (Hemerly *et al.* 1995), the ant mutant of *Arabidopsis thaliana* (Mizukami & Fischer 2000), the *ICK1* overexpressor of *A. thaliana* (Wang *et al.* 2000) and the disruptants of GPA1 in *A. thaliana* (Ullah *et al.* 2001) have smaller and narrower leaves than wild type plants. The epidermal cells of these mutants are larger than those of the wild type, but there are

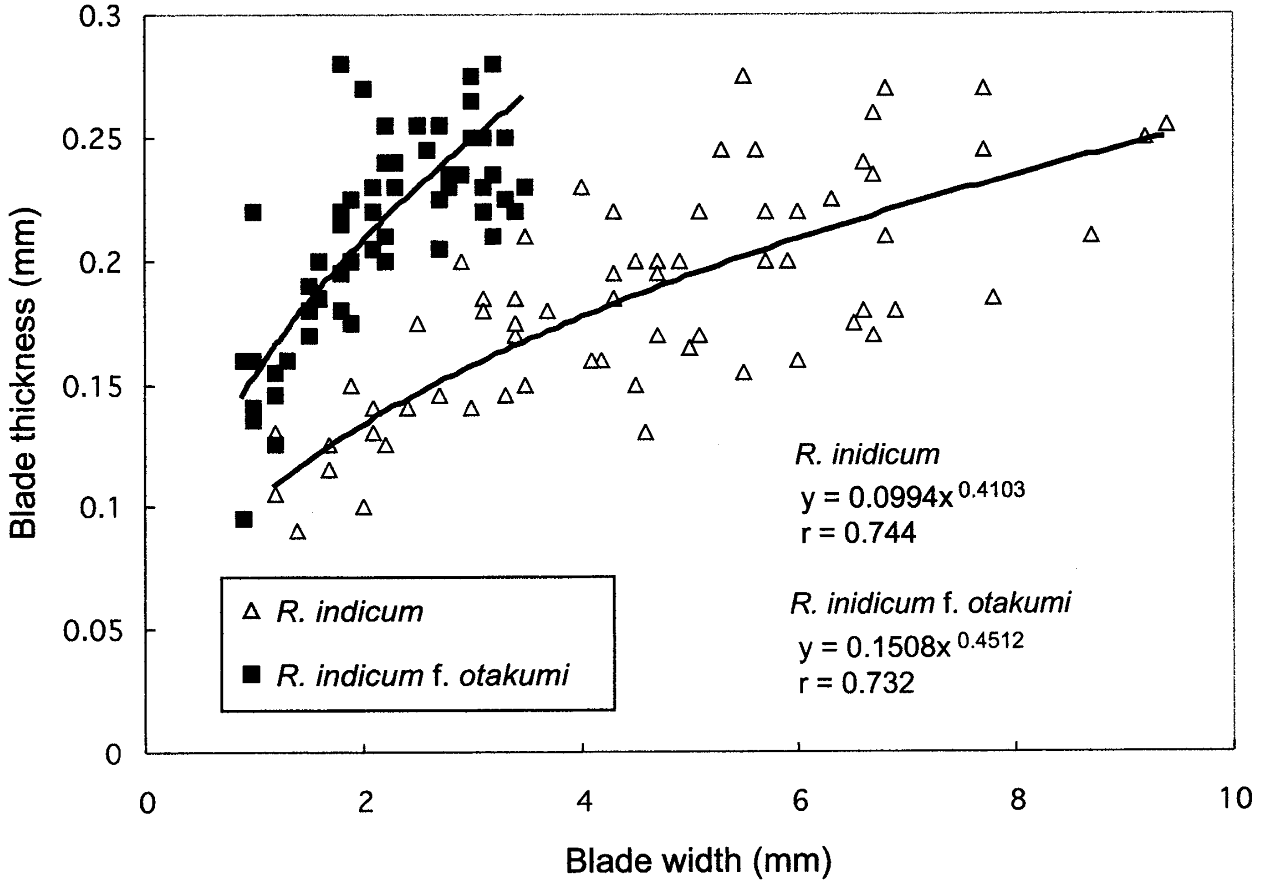

FIG. 6. Correlation between blade thickness and blade width. r is the correlation coefficient

TABLE 2. Dimensions of adaxial epidermal cells and palisade cells, and number of adaxial epidermal cells. Maximum and minimum dimensions / numbers are shown in parentheses (Max./Min.). 161 matured leaves were observed in each taxon.

Plant taxa	Length in the leaf width direction (μm)[a] (Mean ± SD)		Thickness (mm)[a] (Mean ± SD)		Number of adaxial epidermal cell
	Adaxial epidermal cell	Palisade cell	Adaxial epidermal cell	Palisade cell	
R. indicum f. *indicum*	34.33 ± 8.43	11.88 ± 2.51	36.05 ± 7.28	49.48 ± 8.93	196.75±17.32
	(60.0 / 15.0)	(17.5 / 7.5)	(55.0 / 17.5)	(72.5 / 30.0)	(227/175)
R. indicum f. *otakumi*	39.84 ± 9.79	13.06 ± 3.07	39.98 ± 7.36	46.20 ± 8.11	87.00±12.12
	(62.5 / 20.0)	(22.5 / 7.5)	(67.5 / 22.5)	(65.0 / 25.0)	(100/69)

[a] Twenty cells were measured in each leaf.

fewer cells (see Tsukaya 2002b). Thus, the morphological difference in f. *indicum* and the possibly derived rheophyte *R. indicum* f. *otakumi* may be attributable to a similar mechanism brought about through mutations.

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TABLE 3. Comparisons of morphological alterations observed in rheophytes.

	Number of cells		Cell size	
	epidermis	palisade	epidermis	palisade
Osmunda lancea (Imaichi & Kato 1992)	same[a]	same[b]	decreased[a]	decreased[b]
Farfugium japonicum var. *luchuense* (Usukura *et al.* 1994)	slightly increase[d] or same[c]	(-)[d]	slightly decreased or same	(-)[d]
Dendranthema yoshinaganthum, *D. indicum* and their hybrid complex (Tsukaya 2002a)	(-)[d]	decreased	(-)[d]	slightly decreased
Present study	decreased	decreased	slightly increased or same	slightly decreased or same

[a] Number and size of intervein cell rows in adaxial epidermis.
[b] Number and size of intervein cell rows in mesophyll.
[c] Cell number per unit distance.
[d] Data has not been obtained.

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